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REVIEW ARTICLE

On the biological role of histone acetylation

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INTRODUCTION: CONSERVED CHARACTER OF MULTIPLE ACETYLATION POSITIONS OF CORE HISTONES

Organization of the eukaryotic genome and histone structure

The basic structural organization of DNA in chromatin of the eukaryotic cell is the repeating unit of the nucleosome core particle plus linker DNA (Klug *et al.*, 1980). The nucleosome core particle consists of an octamer of core histones with two copies each of histones H2A, H2B, H3 and H4 (core histones) plus 145 base pairs of DNA organized in 1.75 turns around the histone octamer (Finch *et al.*, 1977; Richmond *et al.*, 1984). The nucleosome core particles are connected by linker DNA, the length of which is species-, tissue- and development-dependent. Thus, the repeating unit of chromatin varies between about 150 to 250 base pairs of DNA (Compton *et al.*, 1976; Morris, 1976; Weintraub, 1978; Savic *et al.*, 1985). The bulk of nucleosome core particles is associated with histone H1. The nucleosome core particle plus histone H1 (linker histone) and the entire linker DNA is called the nucleosome (Klug *et al.*, 1980; McGhee & Felsenfeld, 1980; Igo-Kemenes *et al.*, 1982; Klug, 1983). The nucleosome core particle plus histone H1 with the additional 23 base pairs of linker DNA to which histone H1 is bound is called the chromatosome, which thus consists of all the nucleosomal histones and about 168 base pairs of DNA (Simpson, 1978*a*). Histone H1 has been found to be present in all eukaryotic organisms, but in *Saccharomyces cerevisiae* the presence of histone H1 is a matter of controversy. Although an immunological investigation strongly suggests that there are histone H1-like proteins in yeast (Srebreva *et al.*, 1987), in another study it was demonstrated that a 'histone H1-like protein' of *Saccharomyces cerevisiae* is a mitochondrial protein (Certa *et al.*, 1984).

The core histones have a highly conserved primary structure, whereas histone H1 is much less conserved (Isenberg, 1979). It is generally assumed that the nucleosome core particle structure represents the major constraint for the conserved character of core histones. Room for variability in the nucleosome core particle structure is provided nonetheless by primary structure variants and covalent modifications of the core histones.

Since in different tissues of a species the same DNA sequences are correlated with distinctly different programs of gene expression, tissue-specific gene expression could be explained either by the methylation pattern of DNA and/or by tissue-specific chromosomal proteins.

It was discovered by Phillips (Phillips, 1963) that the *N*-terminal nitrogen of histones H1, H2A and H4 is irreversibly acetylated. In most cases examined the acetylated *N*-terminus of histones was identified as a stable N^{α}-acetylserine (Isenberg, 1979). Such an irreversible type of acetylation of the *N*-terminus, taking place simultaneously with protein synthesis, is found also in many cytoplasmic proteins (Brown & Roberts, 1976; Brown, 1979), and there is no indication that it could have any specific role related to the structure and function of the nucleosome core particle.

Reversible histone acetylation, which occurs at the ϵ-amino group of specific internal lysine residues located at the highly basic *N*-terminal domains of core histones, was discovered by Vincent Allfrey and colleagues (Allfrey *et al.*, 1964; for reviews see Allfrey, 1971; Allfrey, 1977; Doenecke & Gallwitz, 1982; Reeves, 1984; Matthews & Waterborg, 1985; Matthews, 1988). In several different systems, including yeast, chicken erythrocytes and HeLa cells, it was observed that post-translational acetylation has *in vivo* a high specificity for core histones and does not occur in histone H1 and nonhistone chromosomal proteins (Nelson, 1982; Zhang & Nelson, 1986). However, reversible acetylation of internal lysine residues of the high-mobility-group nonhistone proteins HMG-1 and HMG-2 in calf thymus (Sterner *et al.*, 1979) and of HMG-14 and HMG-17 in duck erythrocytes (Sterner *et al.*, 1981) was observed. Furthermore, it was reported that the drug doxorubicin caused post-translational acetylation of histone H1 but only in a doxorubicin-resistant human colon cancer cell line and not in the parental cell line (Mannironi & D'Incalci, 1988). No post-translational acetylation could be detected in any other cellular protein of this drug-resistant cell line and the acetylation pattern of core histones remained essentially unaffected by the treatment with doxorubicin (Mannironi & D'Incalci, 1988). It cannot be excluded that in certain physiological situations individual nonhistone chromosomal proteins become targets of post-translational acetylation. This is suggested by studies with liver tissue cultures (Jiakuntorn & Mathias, 1981) and with Chang liver cells (Kaneko, 1983).

The highly conserved character of histone proteins suggests that the entire histone polypeptide chain is indispensable, and the highly conserved positions of acetylation argue for a fundamental function of this modification for the eukaryotic cell. The multiple acetylation positions of core histones represent the potential for an enormous variety of structures and structural isomers of the nucleosome core particle. Moreover, the acetylation of a differing number of nucleosomes located at different chromatin regions is possible and acetylations occur with different turnover. Distinctly different acetylation patterns emerge when species, cell lines, different physiological situations and chromatin fractions are compared. The actual acetylation pattern is determined by the available acetylation positions on the one side, and the activities of histone acetyltransferase(s) and deacetylase(s) on the other. Thus, the regulation of histone acetylation is conceivable either at the level of chromatin structure, or alternatively at the level of

acetylating and deacetylating enzymes which, in this latter case, would be the cause of steric alterations in chromatin. While many histone genes are cloned, sequenced and their organization in the genome elucidated (reviewed by Marzluff, 1986), not much is known about structure, regulation and exact cellular location of the enzymes of reversible histone acetylation.

Histone proteins and their domains differ in their degree of conservation (Isenberg, 1979). Point substitutions occur in the hydrophobic central and *C*-terminal regions of all four core histones. The highest degree of conservation, namely identity, is given in the basic *N*-terminal region of histone H4, in residues 1–20, and of histone H3, in residues 1–30 (DeLange *et al.*, 1969*a*,*b*; Ogawa *et al.*, 1969; Isenberg, 1979; Von Holt *et al.*, 1979; Ohe & Iwai, 1981; Waterborg & Matthews, 1983; Waterborg *et al.*, 1983), with the only known exception of histone H4 of *Tetrahymena* (Glover & Gorovsky, 1979; Hayashi *et al.*, 1980). Since in the case of *Tetrahymena*, in the *N*-terminal region of histone H4, amino acids other than the lysine residues are substituted, inserted and deleted, one can say that, based on current evidence, the positions of acetylation in histone H4 are invariant and are thus conserved to a higher degree than the histone primary structure itself. The basic *N*-terminal domains of histones H2A and H2B are less conserved and show some divergence (Von Holt *et al.*, 1979; Wu *et al.*, 1982; Mende *et al.*, 1983; Vanfleteren *et al.*, 1987).

Specific pattern of histone acetylation

In histone H4, acetylation occurs in all four of the lysine residues of the *N*-terminal domain. Histone H3 was reported to have four to five acetylation positions. Interestingly, in histone H2A only one or two positions are acetylated, and even in hyperacetylated chromatin several lysine residues in the *N*-terminal domain remain nonacetylated. In histone H2B four or five positions can be acetylated, although seven to eleven positions are available.

In the classical experiments of Vincent Allfrey and coworkers, a positive correlation was found between reversible histone acetylation and overall transcriptional activity. In mitogen-stimulated lymphocytes, acetate incorporation into histones increased within a few minutes after stimulation (Pogo *et al.*, 1966). In studies on acetylation of histones using labelled acetate it is often difficult to ensure that increased radioactivity incorporated into histones in response to a given biological stimulus (e.g. hormones) is indeed due to a higher degree of acetylation and not the result of changing pool sizes of acetate, acetyl-CoA and increased uptake of acetate by the cells in question. Regenerating rat liver exhibited an increased rate of acetate incorporation into histones and a decreased rate of acetyl turnover (Pogo *et al.*, 1968). However, soon it was realized that acetylation might have more than one function, since a high steady-state level of acetylation of histone H4 was temporally correlated with the transcriptionally inactive late stage of spermatogenesis in trout (Sung & Dixon, 1970; Candido & Dixon, 1971), and also in trout testis, cytoplasmic reversible acetylation of newly-synthesized histone H4 was detected (Louie & Dixon, 1972). Positions of acetylation were determined in histone H4 of calf thymus (DeLange *et al.*, 1969*a*), pea (DeLange *et al.*, 1969*b*) and trout testis (Candido & Dixon, 1972). In the following studies, evidence continued to accumulate that not only distinct steady-state levels of histone acetylation but also specific patterns of acetylation are correlated with different physiological situations. A decreasing steady-state level of histone acetylation was observed to accompany bird erythrocyte maturation (Ruiz-Carrillo *et al.*, 1974), and in duck erythroid cells, reversible acetylation of newly-synthesized histone H4 was detected (Ruiz-Carrillo *et al.*, 1975). In the yeast *Saccharomyces cerevisiae*, the mono- and tetra-acetylated forms of H4 were identified as the most abundant, especially in the G1 phase (Marian & Wintersberger, 1982). Two distinct types of cell-cycle-dependent histone acetylations were detected in *Physarum* (Waterborg & Matthews, 1984). In the sea urchin *Arbacia punctulata* the relative amount of diacetylated H4 correlates with the rate of cell doubling during early embryogenesis and decreases as the nucleosomal repeat increases, implying a role of diacetylated histone H4 in the maturation of newly replicated chromatin (Chambers & Shaw, 1984).

Nonrandom utilization of multiple acetylation positions

In several species the positions of reversible histone acetylation were identified by amino acid sequencing techniques. In histones H4 and H3 the positions of reversible acetylation are highly conserved. In H4 they are at positions 5, 8, 12, and 16 (in *Tetrahymena* at 4, 7, 11 and 15). Histone H3 can be reversibly acetylated at positions 9, 14, 18, 23 (in some species also at position 4). In H2A and H2B the basic *N*-terminal regions are more variable. Histone H2A is reversibly acetylated at position 5, and in some species also in a second position which is, for instance, position 9 in calf thymus. The acetylation positions of H2B show some divergence, for instance, H2B from calf thymus is acetylated at positions 5, 12, 15 and 20; histone H2B from trout testis at positions 5, 10, 13 and 18 (for reviews see Isenberg, 1979; Doenecke & Gallwitz, 1982; Matthews & Waterborg, 1985).

Recent investigations have shown that the positions of acetylation are utilized nonrandomly. The ciliated protozoan *Tetrahymena pyriformis* provides the advantage that post-synthetic transcription-related acetylation can be separated from the deposition-related one of newly-synthesized histones, since transcription takes place only in macronuclei and not in micronuclei of this organism. Nucleosomal histones are acetylated to a high extent in transcriptionally active macronuclei, whereas their acetylation is marginal in micronuclei (Gorovsky *et al.*, 1973; Vavra *et al.*, 1982*a*; Allis *et al.*, 1985). In histone H4 of *Tetrahymena*, the amino acid which in other species is in position 3 (arginine) is deleted, and the acetylation positions of histone H4 were identified in lysines at positions 4, 7, 11 and 15 (Chicoine *et al.*, 1986). Lysine 7 was identified as the exclusive position of post-synthetic acetylation in monoacetylated H4 isolated from macronuclei. In diacetylated H4, in addition to lysine 7, lysine 4 is also acetylated, and there is preference for lysine 11 as the third position of acetylation in triacetylated H4 molecules. However, in newly-synthesized diacetylated H4 the positions 4 and 11 are used exclusively, regardless of whether H4 is destined for macro- or micro-nuclei. Macronuclear H3 of *Tetrahymena* contains acetylation positions at lysines 9, 14, 18 and 23; in monoacetylated H3 either position 9 or 14 is acetylated, leading to a diacetylated H3 in positions 9 and 14; there is preference for lysine 18 as the third acetylation position (Chicoine *et al.*, 1986).

In the true slime mould *Physarum polycephalum*, which has no G1 phase, the natural synchrony enables one to distinguish between acetylations specific for S phase and G2 phase, respectively. In *Physarum*, histone H4 from growing cells was found to be acetylated at lysines 5, 8, 12 and 16 (Pesis & Matthews, 1986); in G2 phase the greatest turnover was measured on lysine 8, whereas in S phase turnover was greatest on lysine 5 (Matthews, 1988).

A nonrandom sequence of acetylation was also demonstrated in higher eukaryotes, namely in cuttlefish testis and calf thymus (Couppez *et al.*, 1987). In most species at late spermatogenesis protamine-displacement is correlated with a high steady-state level of histone acetylation (Christensen & Dixon, 1982; Christensen *et al.*, 1984; Oliva & Mezquita, 1982; Grimes & Henderson, 1984*a*,*b*). In cuttlefish, differentiation of the spermatozoa takes place in the genital tract, and testis chromatin, being free of protamines, is an excellent starting material for the purification of tri- and tetra-acetylated histone H4 (Wouters-Tyrou *et al.*, 1981). In histone H4 of cuttlefish testis, lysine 12 is the main position of acetylation in the monoacetylated subfraction; lysines 5 and 12 are found acetylated in diacetylated H4; lysines 5, 12, and 16 are acetylated in triacetylated H4; lysines 5, 8, 12, and 16 are acetylated in tetra-acetylated H4. From the stoichiometry of the distinctly acetylated H4 subfractions, it was concluded that lysine 5 is acetylated after lysine 12 (Couppez et al., 1987). In H4 from calf thymus, lysine 16 is the only position of acetylation in the monoacetylated subfraction; all the diacetylated forms are acetylated in lysine 16, the second position of acetylation being, in decreasing order, lysine 12, lysine 5 and lysine 8, suggesting that acetylation occurs in a sequential manner (Couppez *et al.*, 1987).

These studies have shown that distinct patterns arising from a nonrandom utilization of the multiple acetylation positions are correlated with different physiological events such as DNA replication-related deposition of newly-synthesized histones, transcriptional activation of genes, and protamine-displacement. However, the exact role of histone acetylation in these processes is not known.

High-resolution p.m.r. investigation of nucleosome core particles revealed that the basic *N*-terminal and *C*-terminal domains of H2A and H2B are not firmly bound to DNA in nucleosome core particles (which do not contain linker DNA) and exhibit mobilities close to random coil conformation. Thus, one can assume that these domains of the histones H2A and H2B interact with linker DNA (Cary *et al.*, 1978). An increase of the ionic strength to 0.6 M-NaCl causes, in addition, the release of the *N*-terminal domains of the histones H3 and H4 which, on the other hand, shows that the *N*-terminal tails of histones H3 and H4 do bind to nucleosome core particle DNA but with a lower affinity than the hydrophobic central and the *C*-terminal domains of these histones (Cary *et al.*, 1978). Interestingly, extensive hyperacetylation of H2A and H2B, with a very limited effect on H3 and H4, was achieved by a mild chemical acetylation of rat liver chromatin (Csordas *et al.*, 1984). The *N*-terminal tails of all core histones can be selectively removed by limited proteolytic cleavage of nucleosome core particles, suggesting that they are the most exposed protein domains of the nucleosome core particle (Weintraub & Van Lente, 1974; Whitlock & Simpson, 1977; Lilley & Tatchell, 1977). As the positions of acetylation are located at the *N*-terminal domains of core histones (for reviews see Isenberg, 1979; Doenecke & Gallwitz, 1982; Matthews & Waterborg, 1985), and these domains are not responsible for the major histone–DNA interactions stabilizing the nucleosome core particle, the idea that acetylation by neutralization of the positive charge of the ϵ-amino group of lysine residues leads to a relaxation of DNA–histone interactions within the nucleosome core particle, is not obvious. The point to be stressed in the present review is that acetylation, not only of newly-synthesized histones H3 and H4 (Sealy & Chalkley, 1979) but also of nucleosomal histones, may be related to the pathway of histones not bound to DNA. Thus, the acetyl group might be just a 'tag' which has to be put on nucleosomal histones before their displacement as a requirement for the redeposition pathway, and, in the case of protamine-displacement perhaps, to promote proteolytic degradation of displaced histones.

As a changing pattern of histone acetylation accompanies various events associated with disassembly and reassembly of chromatin, it needs to be examined whether, and to what extent if at all, chromatin structure is altered by the acetylation of histones.

HISTONE ACETYLATION AND CHROMATIN STRUCTURE

Histone acetylation might affect chromatin structure at least at three different levels. First, acetylation of newly-synthesized histones is related to the formation of nucleosomes via the histone deposition pathway. Second, acetylation of nucleosomes might have a direct consequence for the nucleosome core particle structure itself, and third, for the higher order structure of chromatin. Not only the formation of nucleosomes from newly-synthesized histones but also reconstitution of nucleosome core particles from displaced histones, and thus also to some extent the reassembly of the higher order structure of chromatin, could be related to the role of acetylation in the processing and deposition of displaced histones.

HISTONE ACETYLATION AND THE HISTONE ASSOCIATION–DISSOCIATION EQUILIBRIUM

Newly-synthesized histones H3 and H4 are reversibly acetylated and become deacetylated after being organized in nucleosomes (Candido & Dixon, 1972; Ruiz-Carillo *et al.*, 1975; Jackson *et al.*, 1976; Sealy & Chalkley, 1979; Woodland, 1979; Cousens & Alberts, 1982). However, prompt deacetylation of histones H3 and H4 does not appear to be a prerequisite for the deposition process (Chalkley and Shires, 1985). In a recent study, the effect of acetylation on the conformation of histones and on their tendency to aggregate was investigated (Prevelige & Fasman, 1987). With the techniques of size exclusion chromatography and sedimentation-equilibrium it was shown that acetylated core histones associate to larger and more stable aggregates. C.d. studies demonstrated that the amount of α-helix increases with increasing association of the histones, and acetylation causes an increase in the α-helix content of the histone molecule (Prevelige & Fasman, 1987).

Moreover, hyperacetylation of histones promotes the efficiency of reconstitution to nucleosome core particles

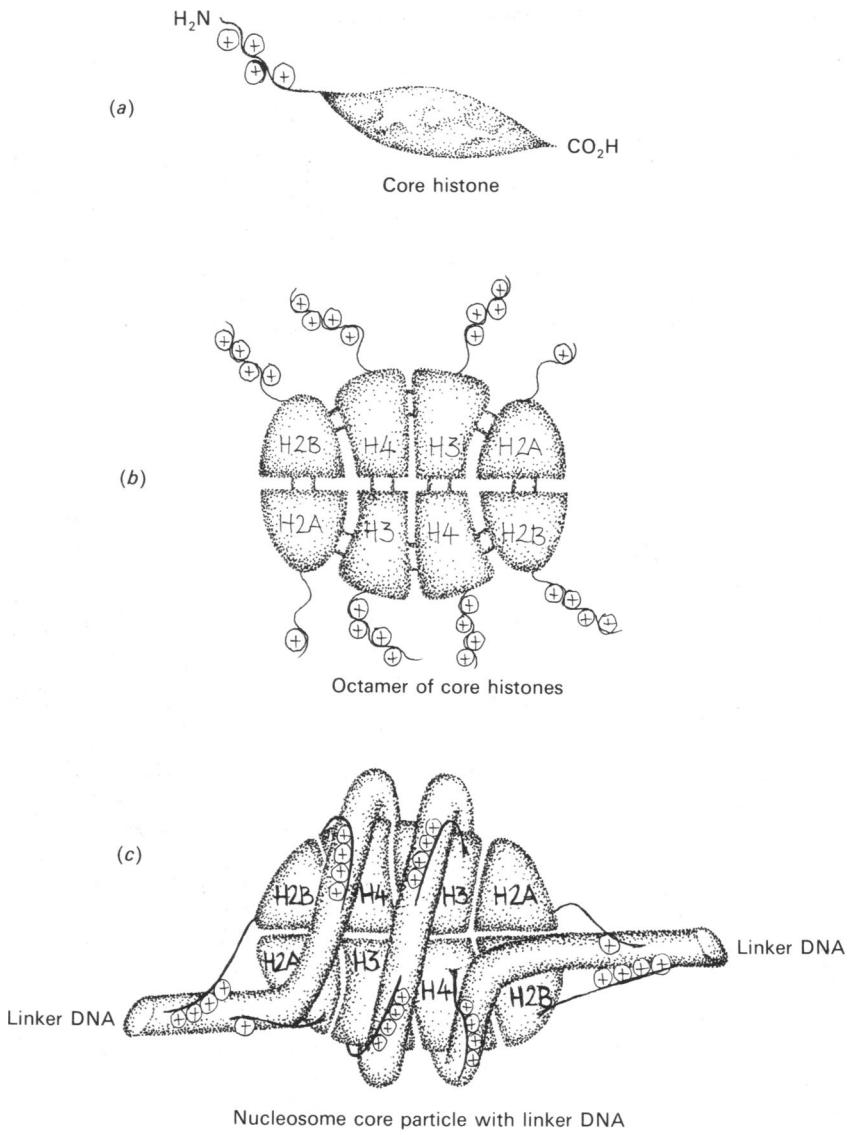

Fig. 1. Schematic illustration of the organization of core histone molecules and their *N*-terminal domains with the positions of acetylation within the nucleosome core particle

(*a*) The cartoon shows the positions of acetylation as positive charges at the *N*-terminal domain ('tail') of the core histone molecule; the positions of acetylation are ϵ-amino groups of lysine residues which are neutralized by acetylation. Additional positive charges resulting from other lysine and arginine residues which do not represent positions of acetylation (at the *N*-terminal domain as well as at the central and *C*-terminal domains) are omitted. Since histone molecules have a certain amount of α-helical content and a distinct conformation, the central domain is drawn as a more compact structure. (*b*) The organization of the octamer of core histones is shown; the *N*-terminal tails and thus the positions of histone acetylation are located at the outer surface of the histone octamer; the *N*-terminal domains are depicted in the deacetylated form and in a random coil conformation. (*c*) Nucleosome core particle plus linker DNA: in the nucleosome core particle, DNA is wound with 1.7 turns around the histone octamer. The *N*-terminal tails of core histones are shown in the non-acetylated form as they interact with the negatively charged DNA and thus reduce the net negative charge; the *N*-terminal domains of histones H3 and H4 interact with DNA within the nucleosome core particle, whereas those of histones H2A and H2B bind to linker DNA adjacent to the nucleosome core particle. The *N*-terminal domains and thus the charges which are neutralized by histone acetylation do not represent the major binding sites of DNA-histone interactions responsible for nucleosome stability; the *N*-terminal tails can be cleaved off by limited proteolysis, while the organization of DNA is retained in the nucleosome core particle fragment.

(Cotten & Chalkley, 1985), the efficiency of reconstitution being correlated with the level of acetylation (Norton *et al.*, 1989). Thus, the neutralization of positive charges at the *N*-terminal domain of core histones favours the stabilization of histone octamers and the formation of nucleosome core particles.

HISTONE ACETYLATION AND NUCLEOSOME CORE PARTICLE STRUCTURE

The effect of histone acetylation on nucleosome core particle structure has been studied by physicochemical, biochemical, immunological and genetic methods. On

the one side, hyperacetylated nucleosome core particles were examined as to their structural alterations when hyperacetylation was achieved by incubating cells with butyrate, an inhibitor of histone deacetylase(s) which does not interfere with the activity of the acetyltransferase(s) (Riggs *et al.*, 1977; Sealy & Chalkley, 1978*a*; Candido *et al.*, 1978; Vidali *et al.*, 1978; Cousens *et al.*, 1979). In other approaches reconstituted nucleosome core particles were investigated as to the conformational changes introduced by acetylated histones (Muller *et al.*, 1982; Norton *et al.*, 1989). In further studies, the contribution of the *N*-terminal sequences of core histones to the conformation and stability of nucleosome core particle was tested after their deletion, either by proteolytic cleavage (Dumuis-Kervabon *et al.*, 1986) or by genetic manipulations (Schuster *et al.*, 1986).

Physicochemical investigations of the conformation and stability of hyperacetylated nucleosome core particles

Incubation of eukaryotic cells with butyrate leads to hyperacetylation of a certain amount of nucleosomal histones but, with the exception of *Saccharomyces cerevisiae*, a substantial proportion of nucleosomal histones remains in the nonacetylated and monoacetylated forms (Cousens *et al.*, 1979). The yield of the hyperacetylated fraction strongly varies with the cell line (Schröter *et al.*, 1981). The term 'hyperacetylation' refers to a level of acetylation above the physiological one, achieved, for instance, by a treatment of the cells with the drug butyrate, whereas the term 'highly acetylated' refers to the nonrandom pattern of an unperturbed physiological situation where the histones happen to be acetylated to a high degree. Although there are 26–32 acetylation positions per nucleosome, the average number of acetyl groups per nucleosome achieved by butyrate treatment of cells is much below this number, since a fraction of nucleosomes is refractory to hyperacetylation.

In hyperacetylated nucleosome core particles from HeLa cells with, on the average, two to three acetyl groups per histone H4, a site located 60 bp from the ends of DNA was found to have greatly increased DNAase I susceptibility, and such core particles exhibited slightly different physicochemical properties (Simpson, 1978*b*). When Chinese hamster ovary cells were hyperacetylated with butyrate up to at least seven acetyl groups per core particle, an altered accessibility of the histone H3 cysteine side-chain was detected with a fluorescence probe specific for thiol groups, which could result from the extension of the α-helical segments in the core histones (Bode *et al.*, 1980). Moreover, a decreased affinity of core histones to DNA of the nucleosome core particle due to hyperacetylation was suggested by changes in the premelting region of the thermal denaturation profile and also by a preferential displacement of histones with protamine (Bode *et al.*, 1980). In other studies, with lymphoblastoid cells hyperacetylated to more than 10 acetyl groups per core particle, and with HeLa cells hyperacetylated to two to three acetyl groups per H4, the hyperacetylated nucleosome core particles exhibited slightly decreased mobilities in non-denaturing nucleoprotein gel electrophoresis, indicative of an increased conformational freedom (Bode *et al.*, 1983; Imai *et al.*, 1986).

Furthermore, the effect of histone hyperacetylation on nucleosome conformation and stability was investigated with nucleosomes from HeLa cells which had up to 17 acetyl groups per nucleosome. The hyperacetylated nucleosome core particles exhibited unchanged stability towards high salt (Ausio & Van Holde, 1986), but exhibited minor differences in their hydrodynamic behaviour and c.d., which could be explained by a partial release of the *N*-terminal tails in hyperacetylated core particles. The previous finding (Simpson, 1978*b*) was confirmed, that as a result of hyperacetylation a site located 60 bp from the ends of DNA in the nucleosome core particle becomes more DNAase I-sensitive (Ausio & Van Holde, 1986) suggesting a subtle conformational change within the 80–100 bp of the nucleosome core particle where histones H3 and H4 have their major contacts with DNA (Richmond *et al.*, 1984). Moreover, in the thermal denaturation profile of the hyperacetylated nucleosome core particles a shift of the first transition from 73 to 70 °C, with a concomitant quantitative increase, was observed. Based on the increased amount of DNA melting in the first transition step, and the DNAase I-sensitive site, it was suggested that acetylation of the *N*-terminal tails of histones H3 and H4 leads to a weakening of histone–DNA interactions in the inner 80–100 bp of the core particle which makes the inner DNA coil more readily displaceable from the histone core (Ausio & Van Holde, 1986). However, it should be pointed out that the unchanged salt sensitivity does not support the idea that hyperacetylated histones, due to their decreased affinity to DNA, are better prepared for displacement. H2A and H2B appear to be the first histones to be displaced from the nucleosomes of actively transcribed genes (Baer & Rhodes, 1983), and, as the *N*-terminal domains of H2A and H2B most likely bind to linker DNA (Cary *et al.*, 1978, 1982), it is not obvious that hyperacetylation affects the binding affinity of H2A and H2B to DNA *within* the nucleosome core particle.

With the technique of neutron scatter it is possible to determine with high accuracy the shape of nucleosome core particles in solution (Hjelm *et al.*, 1977; Suau *et al.*, 1977; Pardon *et al.*, 1977; Braddock *et al.*, 1981). With this technique no sign of unfolding could be detected in hyperacetylated nucleosome core particles with 2.4 acetates per H4 molecule (Imai *et al.*, 1986). Recently it was shown with reconstituted nucleosome core particles that the negative linking number decreases when hyperacetylated histones are used for reconstitution, and it was calculated that this corresponds to an uncoiling of 18.5 bp (Norton *et al.*, 1989). Thus, all the physicochemical and biochemical data agree on the point that histone hyperacetylation itself does not cause a drastic unfolding and disassembly of nucleosome core particles. However, at the highest levels of hyperacetylation achieved by butyrate treatment, minor changes in the conformation of the core particles have been detected. These conformational changes are most likely due to hyperacetylated histones, but one has to bear in mind that butyrate has manifold pleiotropic effects (reviewed by Kruh, 1982) such as, for instance, increased susceptibility of histone H3 to calcium-dependent phosphorylation (Whitlock *et al.*, 1980), and decreased phosphorylation of H2A (Boffa *et al.*, 1981).

A key question concerning the biological role of histone acetylation is, to what extent the minor conformational changes, manifested as slightly altered physicochemical parameters and as the additional DNAase I-sensitive site in hyperacetylated nucleosome core particles, are functionally relevant. However, currently there is no evidence

which would link the observed conformational change of hyperacetylated nucleosome core particles in a causal manner to a specific biological function.

Because of the high affinity of histones to DNA, they were originally considered to be the general repressors in eukaryotic gene regulation, and acetylation of histones appeared to be a mechanism of derepression, since neutralization of positive charges could be expected to decrease the binding affinity of core histones to DNA. However, recent experiments *in vitro* have shown that nucleosomal core histones do not inhibit the *elongation* event of transcription, as the elongation rate on naked DNA and on the same DNA sequence in a nucleosome core particle was found to be identical (Lorch *et al.*, 1987, 1988) or almost identical (Losa & Brown, 1987), but *initiation* of transcription is inhibited when the promoter sequence is positioned within a nucleosome core particle (Lorch *et al.*, 1987; Workman & Roeder, 1987). Thus, hyperacetylation of histones is not an absolute requirement *in vitro* for the elongation event of transcription of DNA organized in nucleosome core particles (Lorch *et al.*, 1987, 1988; Losa & Brown, 1987; Workman & Roeder, 1987; Workman *et al.*, 1988), and displacement of core histones due to the elongation event of transcription takes place *in vitro* without the need for a preceding acetylation of nucleosomal histones (Lorch *et al.*, 1987, 1988). Moreover, it has also been shown that binding of the transcription factor TFIID to a promoter sequence prior to nucleosome assembly prevents the repression of transcription-initiation by histones (Workman & Roeder, 1987). In the light of these experiments, histone acetylation as a mechanism of transcriptional derepression, at the level of the nucleosome core particle structure, can be postulated only as a requirement for the destabilization of those nucleosomes which are located at promoter sequences in order to enable the binding of transcription factors. However, such a role would affect at best a single nucleosome per gene in a transient manner, thus accounting only for a hardly detectable low steady-state level of histone acetylation, since there would be no need for nucleosomal acetylation in further rounds of transcription. The high degree of acetylation found in nucleosomal histones of active genes (Allegra *et al.*, 1987; Hebbes *et al.*, 1988; Ip *et al.*, 1988), the high steady-state level (Gorovsky *et al.*, 1973), and the high turnover of acetylation (Ip *et al.*, 1988; Zhang & Nelson, 1988) in transcriptionally active nuclei can hardly be accounted for exclusively by the event of establishing a derepressed promoter. A recent study revealed that an *in vivo* transcriptional activator, the immediate early protein of pseudorabies virus, in the presence of TFDII potentiates the activity of the adenovirus major late promoter by competing with histones during the formation of nucleosomes *in vitro* (Workman *et al.*, 1988). However, the transcriptional activator is able neither to reverse repression once an inactive nucleosome is positioned on a promoter sequence nor to further increase the activity of previously reconstituted TFIID-containing active nucleosomes on a promoter sequence (Workman *et al.*, 1988). Thus, the transcriptional activator competes with histones by increasing the probability of TFIID binding, and/or by decreasing the probability of histone binding to promoter sequences. It is conceivable that the efficiency of assembly of potentially active preinitiation complexes can be affected, on the one side, by the molar amount and modification of the transcription factors, and on the other, by modification of histones, by specific DNA sequences and by DNA-methylation. Since acetylation was shown to interfere with aggregation (Prevelige & Fasman, 1987) and deposition (Cotten & Chalkley, 1985; Norton *et al.*, 1989) of histones, it appears as one of the factors which enables the subtle tuning of binding affinities in a multiple equilibrium leading to differentiation and tissue-specific gene expression. However, considering the extent and dynamics of acetylation of nucleosomes associated with transcriptionally active genes, the function of histone acetylation cannot be confined to the formation of 'potentiated' nucleosomes permitting initiation of transcription.

Biochemical, immunological and genetic analyses related to the function of acetylation of histones in nucleosomes

Limited proteolysis of nucleosome core particles. Nucleosomal fragments can be prepared by limited proteolysis, in which the core histones are devoid of their *N*-terminal tails, i.e. of their acetylation positions. This offers an approach to testing the contribution of these domains to the stability of the nucleosome core particle structure. It was observed that the loss of 10–30 amino acids at the *N*-termini of core histones after limited digestion of core particles with trypsin does not lead to any drastic change in the nucleosome core particle structure (Weintraub & Van Lente, 1974; Böhm *et al.*, 1981; Palter & Alberts, 1979; Whitlock & Stein, 1978; Grigoryev & Karsheninnikov, 1982). After limited proteolysis of chicken erythrocyte nuclei with trypsin, only minor destabilization of the nucleosome structure was observed (Grigoryev & Karsheninnikov, 1982). A recent study has shown that proteolysis with the enzyme clostripain yields nucleosome core particle fragments with a higher degree of isomorphism than that which can be achieved with trypsin. After limited proteolysis of core particles or H1-depleted oligonucleosomes from rat liver with clostripain, histones H2A, H2B, H3 and H4 were selectively cleaved at the carboxyl side of Arg-11, Lys-20, Arg-26 and Arg-19, respectively, while the *C*-terminal sequences remained unaffected (Dumuis-Kervabon *et al.*, 1986). Despite the loss of the highly basic *N*-terminal regions, including approx. 17% of the total amino acids, the characteristic structural organization of the nucleosome core particle was retained and by physicochemical and biochemical criteria only minor, if any, conformational changes could be detected. However, the nucleosome core particle fragments showed an increased binding of spermidine (Dumuis-Kervabon *et al.*, 1986), which suggests that at least some of the cleaved-off *N*-terminal lysine and arginine residues interacted with the phosphate of DNA without making any major contribution to the conformational organization of the nucleosome core particle. Such a weak binding of the *N*-terminal tails to DNA is also suggested by an increased nuclease sensitivity of core particle DNA after limited proteolysis detected in earlier studies (Lilly & Tatchell, 1977; Whitlock & Simpson, 1977). Moreover, the weak interaction of the *N*-terminal domains of histones H3 and H4 with core particle DNA was also demonstrated in an n.m.r. study (Cary *et al.*, 1978).

In the light of the proteolysis experiments, the nucleosome core particle structure itself does not appear to be an evolutionary constraint responsible for the highly conserved character of the *N*-terminal tails of core histones. One could speculate that the binding site of

histone H1, i.e. the structure of the chromatosome, internucleosomal interactions within precisely determined distances of the higher order structure of chromatin and/or conserved motifs of acidic nuclear proteins might represent such constraints. Certainly, an understanding of these constraints appears to be the key to an understanding of the biological role of histone acetylation.

Histone displacement by protamine as a test for histone–DNA binding affinity. In several species, for instance in trout, histones are highly acetylated when displaced by protamine at the late stages of spermatogenesis (Christensen & Dixon, 1982; Christensen *et al.*, 1984). However, in a few other species, as for instance in carp, there is no protamine displacement at late spermatogenesis and there is also no increased steady-state level of histone acetylation (Christensen *et al.*, 1984). An obvious assumption would be that acetylation of nucleosomal histones is the causal prerequisite for histone displacement occurring at late spermatogenesis.

The effect of hyperacetylation on the binding affinity of core histones to DNA was tested by protamine displacement *in vitro*, and a preferential release of histones from the nucleosome core particles of butyrate-treated Chinese hamster ovary cells was observed (Bode *et al.*, 1980). Butyrate-hyperacetylated monomeric nucleosome core particles from HeLa cells were tested, with the result that histones displaced by protamine had a higher degree of hyperacetylation than the residual histones (Oliva *et al.*, 1987). However, protamines proved to be quite efficient also in disassembling control and chicken erythrocyte core particles (Oliva *et al.*, 1987). Since, in trout, spermatogenesis occurs over several weeks, the high steady-state level of histone acetylation does not appear to be mandatory for protamine-mediated histone displacement. Thus, mechanisms other than histone acetylation, such as, for instance, topoisomerase activity could also be responsible for an altered chromatin structure leading to the displacement of histones by protamine, and acetylation could have occurred as a secondary event, being thus not the cause but rather the consequence of the altered chromatin structure. For the understanding of the mechanism of histone displacement by protamine *in vivo*, other highly ordered biochemical events temporally correlated with histone acetylation at late stage of spermatogenesis, such as phosphorylation of newly-synthesized protamines, dephosphorylation of protamines bound to DNA (Louie & Dixon, 1972), and the controlled proteolysis of displaced histones (Sung & Dixon, 1970; Marushige & Dixon, 1971; Christensen & Dixon, 1982), also have to be considered. Interestingly, in trout the nonhistones are also displaced to a considerable extent in the nucleoprotamine fraction but the displacement of histones is incomplete, as the nucleoprotamine fraction always contains a small amount of histone (Marushige & Dixon, 1971). *In vivo*, histone H4 is the first, and histone H1 the last, histone to be displaced from trout testes chromatin, while *in vitro*, by the criteria of differential salt extraction and displacement by protamine, the opposite order of binding affinities is observed (Marushige & Dixon, 1969, 1971; Christensen & Dixon, 1982). Therefore, it is fair to assume that the mechanism of histone displacement by protamine *in vivo* is a complex one, and is not solely under the control of those electrostatic DNA–histone interactions which determine the order of binding affinities in the nucleohistone *in vitro*. Although in most mammals the displacement of histones by protamine is thought to be complete, in humans approx. 15% of histones remain associated with sperm DNA (Tanphaichitr *et al.*, 1978). In the chromatin of the human spermatozoon, histones and protamines respectively are distributed in a DNA-sequence-specific manner (Gatewood *et al.*, 1987); all four core histones were found to be present, extractable with 0.65 M-NaCl, with high levels of acetylation of H3 and H4 (Gatewood *et al.*, 1987), the degree of acetylation being comparable to that of the histones during spermatogenesis in trout (Christensen & Dixon, 1982). Thus, in human spermatids a high steady-state level of acetylation is not necessarily a signal for their displacement, and the fate of highly acetylated histones is determined in a DNA-sequence-dependent manner.

Immunological analysis of structural changes in acetylated nucleosome core particles. In macromolecular assemblies epitopes can also arise from the juxtaposition of different molecules. Thus, in the nucleosome core particle, peptide chains of different histones associated to stable conformations can form epitopes. Therefore, antibodies are sensitive tools for detecting changes in histone–histone and histone–DNA interactions. In such an approach, antisera specific to H2A, H2B and H3, respectively, were used as probes to detect an altered hapten resulting from conformational changes in nucleosomes (Muller *et al.*, 1982). Reconstituted nucleosome core particles from chicken erythrocytes were compared as to their antigenic determinants with reassociated and native ones, and no significant differences could be detected with the antisera to histone H2A, H2B and H3, respectively. However, when di- or tri-acetylated H4 from cuttle fish testis was used for reconstitution, the binding affinity of antibodies specific for H2A and H3 to the reconstituted core particles was considerably decreased, whereas the binding affinity of antibodies specific for H2B remained the same. The most pronounced effect was observed with the antiserum specific for histone H3, suggesting conformational changes in the H3–H4 regions of the core particle due to the presence of higher acetylated H4 (Muller *et al.*, 1982). It should be pointed out that, in these reconstitution experiments, histone H4 from cuttle fish testis with a characteristic non-random pattern of acetylation was used, and moreover, the observed conformational change of the reconstituted nucleosome core particle is linked to the modification of histone H4. The conformational change due to acetylated H4 appears to be similar to that observed with physicochemical techniques in hyperacetylated nucleosome core particles from butyrate-treated cells.

Genetic deletion of *N*-terminal sequences of core histones. In yeast, the biological function of the *N*-terminal domains of core histones can also be investigated by genetic manipulations (Schuster *et al.*, 1986). It was found that histone H2B protein can function even with large deletions of its *N*-terminus. However, a deletion removing a large portion of the *C*-terminus was lethal (Wallis *et al.*, 1983). In yeast, H2A can complement the deleted *N*-terminal tail of histone H2B. However, deletions at the *N*-termini of both H2A and H2B are lethal

(Schuster *et al.*, 1986). Moreover, H2A cannot substitute for the complete H2B histone protein. After repression of histone H2B synthesis a full round of DNA replication with active transcription and induction of new transcripts continues, but the cells are arrested in mitosis (Han *et al.*, 1987). Repression of H4 synthesis resulted in arrest in the G2 phase, and extensive loss of nucleosomes was observed (Kim *et al.*, 1988). In yeast, the regulation of the POH5 gene encoding an acid phosphatase can be looked upon as an exception, since this gene, unlike other genes of yeast, has a promoter sequence positioned in a nucleosome (Almer & Hörz, 1986). Nucleosome removal is required for the transcriptional activation of the POH5 gene, which is repressed when the concentration of inorganic phosphate is high in the medium, and is induced under conditions of low inorganic phosphate concentration (Oshima, 1982). In cells with extensive nucleosome loss due to histone H4 depletion, the POH5 gene was no longer repressible. It should be pointed out that in a topoisomerase I-deficient strain of yeast with a temperature-sensitive topoisomerase II, the POH5 gene was found to be noninducible at the restrictive temperature (Han *et al.*, 1988), suggesting that acetylation alone is not enough to enable nucleosome removal by transcription factors and/or RNA polymerase II. In yeast there is a high steady-state level of histone acetylation, and assuming that acetylation of nucleosomes occurs at any sterically available chromatin region, it appears unlikely that acetylation could specifically regulate the removal of a single nucleosome.

Recently, yeast strains have been constructed with partial deletions in histone H4 (Kayne *et al.*, 1988). Deletions in the hydrophobic core-domain of H4 block chromosome segregation and are lethal. In contrast, deletions at the *N*-terminus (residues 4–28) and *C*-terminus (residues 100–102) are viable. Thus, most of the *N*-terminus and a short *C*-terminal sequence of histone H4 are dispensable for growth. The consequence of the *N*-terminal deletion was a longer cell-cycle, with an especially longer G2 phase, and a chromatin with increased micrococcal nuclease sensitivity. Most interestingly, there was also a specific effect, namely the derepression of two silent mating type loci (HMLα and HMRa) causing a loss of mating ability (Kayne *et al.*, 1988). However, in this mutant with the deleted *N*-terminus, several other regulated genes tested (including the POH5 gene) were repressed and induced normally, suggesting that the positive charges at the *N*-terminal domain of H4 (which in the wild-type arise from the deacetylation of H4) are not essential for gene repression, at least in the special case of yeast.

The specific function of the *N*-terminal domain of histone H4 concerning the regulation of the two mating type loci (HMLα and HMRa) could possibly be explained by the interaction of the basic *N*-terminal domain with specific regulatory proteins which contain a cluster of acidic residues (Kayne *et al.*, 1988). Such a function would be consistent with the idea put forward in the present review that acetylation of histones is not only of relevance for their binding affinity to DNA but also for the modulation of histone interactions with acidic proteins of the nucleus.

HISTONE ACETYLATION AND THE HIGHER ORDER STRUCTURE OF CHROMATIN

The question whether histone acetylation has an effect on the higher order structure of chromatin has been the subject of intensive study. The condensed higher order structure of chromatin, the solenoid, also called the 30 nm fibre (Finch & Klug, 1976; Sperling & Klug, 1977; Renz *et al.*, 1977; Widom & Klug, 1985; Felsenfeld & McGhee, 1986; Butler, 1988), has about six nucleosomes per turn of the 'beads on a string', the 10 nm filament, and represents a roughly 50-fold contraction of DNA relative to the unperturbed double helix. It was shown that histone H1 is required for the formation of the

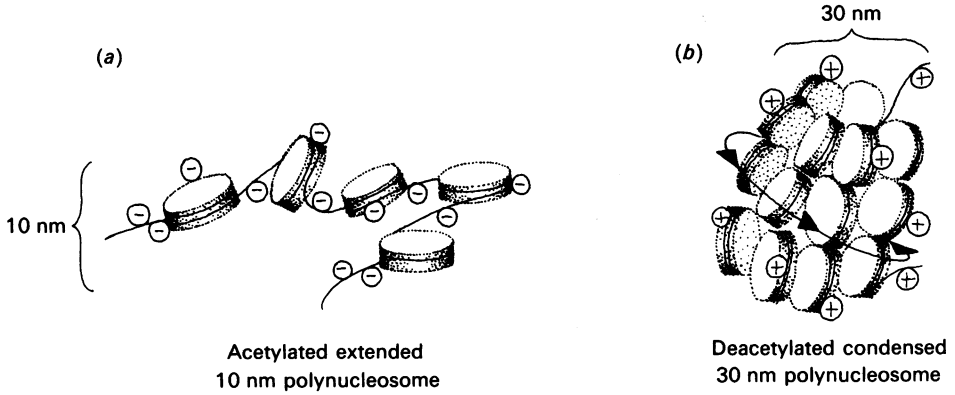

Fig. 2. The effect of histone acetylation on the polynucleosomal organization in the higher order structure of chromatin

(*a*) The extended 'beads-on-a-string' 10 nm-polynucleosomal structure of active and potentially active chromatin might be stabilized by reversible acetylation of core histones by which up to 26–32 positive charges per nucleosome can be neutralized and thus the net negative charge per nucleosome increases considerably. This is indicated in the cartoon by the negative charges within and adjacent to the nucleosome core particles. The repulsive forces of the increased net negative charge favour a more extended conformation of the polynucleosomes. (*b*) Deacetylation, on the other hand, by producing up to 26–32 positive charges per nucleosome, decreases the net negative charge of the nucleosome, which favours a more compact packaging of polynucleosomes within the 30 nm higher order structure of chromatin. The schematically shown positive charges of deacetylated lysine residues lead to a decrease of the net negative charge in a deacetylated polynucleosome.

solenoid (Thoma *et al.*, 1979; Renz *et al.*, 1977; Allan *et al.*, 1981). The condensed higher order structure of chromatin can be expected to be relaxed during transcription and replication.

The effect of histone acetylation on the higher order structure of chromatin was investigated with the techniques of analytical ultracentrifugation, electric dichroism (McGhee *et al.*, 1983), light scattering and flow linear dichroism (Dimitrov *et al.*, 1986). It was shown that hyperacetylation of histones achieved by treatment of HeLa cells with 10 mM-butyrate does not unfold the 30 nm higher order structure to the 10 nm 'beads on a string' chromatin structure (McGhee *et al.*, 1983). Hyperacetylated chromatin was essentially not different from control chromatin in the cation-induced folding of the 10 nm filament to the 30 nm solenoid conformation, from which can be concluded that hyperacetylation of histones in itself does not represent a mechanism for altering the higher order chromatin solenoid (McGhee *et al.*, 1983; Dimitrov *et al.*, 1986).

An interaction of the *N*-terminal domains of core histones with DNA within the nucleosome core particle and on linker DNA is suggested by a thermal denaturation study in which the melting behaviour of control and hyperacetylated mono-, di- and tri-nucleosomes has been compared (Yau *et al.*, 1982). The melting of mononucleosomes is characterized by a premelt and a major co-operative transition, whereas di- and tri-nucleosomes exhibit, in addition, an intermediate transition temperature. Hyperacetylation did not alter the profile of the premelt region, but reduced to a minor but significant extent the temperatures of the major co-operative and intermediate transitions (Yau *et al.*, 1982).

Thus, one function of histone acetylation related to transcription could be an additional relaxation and stabilization of the polynucleosomal configuration of those partially relaxed regions of chromatin which are not condensed in the 30 nm solenoid superstructure and are presumably to a large extent free of histone H1. However considering that by c.d. no structural change could be detected in hyperacetylated H1-depleted chromatin (Reczek *et al.*, 1982; Dimitrov *et al.*, 1986), the effect of acetylation on polynucleosomal configuration can be, if at all, only minor. On the other hand, in a concerted mechanism with histone H1, histone *deacetylation* could promote the folding of polynucleosomes into the condensed higher order structure of the solenoid, by shielding the negative charge of DNA at defined positions. This is suggested by an experiment in which H1- and H5-stripped polynucleosomes were subjected to limited digestion with trypsin in order to remove the *N*-terminal domains of the nucleosomal core histones (Allan *et al.*, 1982). When such polynucleosomes were reconstituted with histone H1, they remained unfolded under conditions otherwise favourable for the formation of solenoids. However, when a basic polypeptide fragment was added, it formed a complex with the H1-reconstituted polynucleosomes, and folding to compact chromatin became possible (Allan *et al.*, 1982). An involvement of the *N*-terminal domains of core histones in the modulation of the affinity of the histone H1 binding site is also indicated in a recent study which suggests that histone acetylation has the ability to release negative supercoils (Norton *et al.*, 1989), and a release of negative supercoils would be expected to alter the geometry of the H1-binding site.

THE ACETYLATION STATUS OF NUCLEOSOMES IN TRANSCRIPTIONALLY ACTIVE CHROMATIN

Altered chromatin structure detected by differential accessibility to nucleases

Since active chromatin is sterically better accessible for nucleases, sequences of active and potentially active genes are cut faster by nucleases than those of inactive ones, and are enriched in solution after limited digestion of nuclear DNA. Such a preferential cutting of active gene sequences was achieved with DNAase I which also cuts DNA within the nucleosome core particle (Weintraub & Groudine, 1976; Garel & Axel, 1976) as well as with micrococcal nuclease and DNAase II, both of which primarily cut between nucleosomes (Gottesfeld & Butler, 1977; Johnson *et al.*, 1978; Mathis *et al.*, 1980). Key questions related to the biological role of histone acetylation raised frequently in this context are first, whether nucleosomes found in the fraction of active genes have a higher degree of acetylation and second, whether hyperacetylation achieved by incubating cells with butyrate would lead to the activation of genes and to an increased amount of the fast-digestible chromatin. Studies with DNAase I (Sealy & Chalkley, 1978*a*,*b*; Nelson *et al.*, 1979; Davie & Candido, 1980; Mathis *et al.*, 1978, 1980; Perry & Chalkley, 1981; Weisbrod, 1982), DNAase II and micrococcal nuclease (Davie & Candido, 1978; Kuehl *et al.*, 1980; Hutcheon *et al.*, 1980) have shown that nucleosomes released in the early digest are enriched in higher acetylated histones. While nuclear DNA of butyrate-treated cells is digested by DNAase I more rapidly than DNA of control cells, core particles from hyperacetylated chromatin are digested at roughly the same rate as the control (Simpson, 1978*b*).

However, in agreement with numerous subsequent studies using various cell types, it was shown that butyrate treatment does not lead to a general activation of genes (Rubenstein *et al.*, 1979). Although an increased level of acetate incorporation into histones was observed to take place as an early event after hormonal stimulation (Libby, 1968, 1972, 1973; Takaku *et al.*, 1969; Pasqualini *et al.*, 1983; Csordas *et al.*, 1986), steroid-hormone-dependent activation of genes, as for instance, egg white mRNA induction in chick oviduct, is inhibited by butyrate (McKnight *et al.*, 1980). Moreover, a lack of a direct causal correlation between highly acetylated histones and the structure of active chromatin was demonstrated in *Tetrahymena*, as deacetylation of macronuclei *in vitro* did not alter the digestion rate of active chromatin (Vavra *et al.*, 1982*b*).

In growing yeast all the genes are active or potentially active, the entire chromatin is highly acetylated and also susceptible to fast digestion by DNAase I (Lohr & Hereford, 1979; Davie *et al.*, 1981). If an increase in the steady-state level of histone acetylation is the cause and not the effect of an altered chromatin structure, the same high percentage of DNAase I-digestible chromatin could be expected in other cells also as a result of butyrate treatment. However, in chicken erythrocytes, only a small fraction of histones becomes hyperacetylated as a result of butyrate treatment (Brotherton *et al.*, 1981; Zhang & Nelson, 1986). Experiments *in vivo* using butyrate have to be interpreted with caution because of the manifold pleiotropic effects of this drug (Kruh, 1982). Nevertheless, the fact that in many cell types only a small

fraction of nucleosomal histones becomes hyperacetylated by butyrate treatment strongly suggests that the steady-state level of histone acetylation is not under the sole control of the acetyltransferase/deacetylase equilibrium. In *Saccharomyces cerevisiae* the presence of histone H1 is controversial (Certa *et al.*, 1984; Srebreva *et al.*, 1987), and there is no conclusive evidence for solenoidal chromatin structure (Certa *et al.*, 1984). Therefore the high level of histone acetylation in this organism appears to be consistent with the interpretation that histone acetylation is limited to the relaxed structural domains of chromatin which are free of H1 (and H5). Thus, the nucleosomes associated with histones H1 (and/or H5) in the solenoid appear to be sterically blocked for histone acetyltransferase. Those polynucleosomes from chicken erythrocytes, which are soluble in 0.15 M-NaCl, are enriched with higher acetylated core histones (Ridsdale & Davie, 1987) and are free of H1 and H5 (Hebbes *et al.*, 1988); the same fraction is also enriched with active gene sequences, whereas inactive genes are found in the aggregation-prone 0.15 M-NaCl-insoluble fraction of polynucleosomes (Ridsdale & Davie, 1987; Hebbes *et al.*, 1988).

Thus it appears that only after the more extended structure of active chromatin is established by a tissue-specific mechanism do nucleosomes of this chromatin region become targets of histone acetylation. However, the utilization of acetylation positions of the sterically available nucleosomes is highly specific, as distinct patterns of histone acetylation were found to be associated with different physiological situations. How the specificity of the nonrandom sequential utilization of acetylation positions is achieved *in vivo* is not clear, since most of the purified histone acetyltransferase(s) were shown to acetylate histone substrates apparently according to sterical availability of lysine residues, without any specificity for acetylation positions (Cano & Pestaña, 1979; Estepa & Pestaña, 1983; Dumuis-Kervabon *et al.*, 1986). On the other hand, a cytoplasmic acetyltransferase isolated from *Drosophila melanogaster* embryos acetylates only *free* histone H4 and only in the positions of reversible acetylation *in vivo*. Moreover, with a single histone acetyltransferase from *Tetrahymena* macronuclei, the nonrandom pattern of deposition-related acetylation was achieved when free histones were used as substrate, whereas with mononucleosomes as substrate the same enzyme led to the nonrandom pattern of transcription-related histone acetylation (Chicoine *et al.*, 1987). Different types of histone acetyltransferases have been detected in eukaryotic cells, chromatin-bound 'type A' activities (Libby, 1978; Sures & Gallwitz, 1980; Böhm *et al.*, 1980; Belikoff *et al.*, 1980; Garcea & Alberts, 1980; Estepa & Pestaña, 1983; Travis *et al.*, 1984) and cytoplasmic 'type B' enzymes (Garcea & Alberts, 1980; Sures & Gallwitz, 1980; Wiegand & Brutlag, 1981; Estepa & Pestaña, 1983). Histone deacetylase(s) might be associated with the nuclear matrix (Hay & Candido, 1983*a*,*b*). However, the enzymology of histone acetylation remains to a large extent an enigma. It is also not clear how the equilibrium of histone acetyltransferase(s) and deacetylase(s) is regulated *in vivo*.

Nucleosomes of active and potentially active genes are highly acetylated

With the technique of Hg–agarose affinity chromatography it was shown that DNA sequences of active genes co-purify with highly acetylated histones H4, H3 and H2B (Allegra *et al.*, 1987; Sterner *et al.*, 1987). In nucleosomes which were isolated from HeLa cells in the exponential growth phase, high levels of tri- and tetra-acetylated histones H3 and H4 were found in the Hg-bound nucleosomes, whereas the unbound nucleosomes were deficient in acetylated histones but enriched in phosphorylated H2A. In an analysis of nucleosomes from synchronized HeLa cells, in S phase, gene sequences of H2A and H4 were detected in the Hg-bound fraction, and no binding to Hg–agarose of these sequences occurred when nucleosomes were purified from cells in G2 phase (Sterner *et al.*, 1987). Furthermore, it was shown that the sequences of the proto-oncogenes c-*fos* and c-*myc* could only be detected in the bound fraction when they were actively transcribed. Rapid and reversible changes in the binding characteristics of nucleosomes to Hg–agarose were found to accompany the activation, repression and superinduction of the murine fibroblast proto-oncogenes c-*fos* and c-*myc* (Chen & Allfrey, 1987).

A direct link between core histone acetylation and active genes was established using an antibody against the epitope N^{ϵ}-acetyl-lysine for the isolation of acetylated nucleosomes from embryonic chicken chromatin (Hebbes *et al.*, 1988). Immunofractionation was performed by incubating chromatin with antibody, and the complexes were isolated by centrifugation after binding to formalin-fixed *Staphylococcus aureus* cells that had protein A in the outer membrane. On probing with gene sequences, antibody-bound chromatin was found to be enriched in the actively transcribed α-globin gene and not enriched in the inactive ovalbumin gene (Hebbes *et al.*, 1988).

Little is known about the distinct structural features of active and potentially active chromatin. After fixation of whole hepatoma cells with formaldehyde, it was possible to separate transcriptionally engaged genes from inactive and potentially active ones by density gradient centrifugation, as transcribed genes moved to a lower density than bulk chromatin (Ip *et al.*, 1988). The lower density characteristic for transcriptionally expressed genes is most likely due to the additional protein of the transcription apparatus, processing enzymes and nuclear matrix to which they become crosslinked by formaldehyde. Histones associated with active chromatin were analysed after the reversal of fixation and found to be highly acetylated by a distinct type of acetylation characterized by rapid turnover (Ip *et al.*, 1988).

An investigation of histone acetylation in chicken erythrocytes revealed that histones in both active and potentially active chromatin are rapidly acetylated, and only in mature erythrocytes is there an additional type of acetylation characterized by slow rate (Zhang & Nelson, 1988).

The fact that highly acetylated histones are associated with active genes does not prove, however, that histone acetylation is a causal prerequisite for transcriptional activation. Although temporally correlated with gene transcription, the acetylation of nucleosomal histones could be nevertheless functionally related to later events which arise as a consequence of transcription, such as the organization and re-deposition of displaced histones.

NUCLEOPLASMIN AND OTHER ACIDIC NUCLEAR PROTEINS WITH SPECIFIC BINDING PROPERTIES FOR HISTONES

Nucleoplasmin, discovered in the eggs of *Xenopus laevis* as an activity which promotes the formation of nucleosomes *in vitro* (Laskey *et al.*, 1977, 1978), is also a major component of the oocyte nucleus (Mills *et al.*, 1980; Krohne & Franke, 1980). Histone complexes with acidic nuclear proteins have been detected in *Xenopus laevis* oocyte nuclei (Kleinschmidt & Franke, 1982; Kleinschmidt *et al.*, 1985; Dilworth *et al.*, 1987). Histones H2A and H2B appear to be selectively bound to nucleoplasmin (Laskey *et al.*, 1978; Earnshaw *et al.*, 1980; Sealy *et al.*, 1986). It was shown that in eggs nucleoplasmin is phosphorylated to a higher extent than in oocytes. The higher phosphorylated form has a superior chromatin assembly activity *in vitro* which can be reduced by phosphatase treatment (Cotten *et al.*, 1986). There is a cluster of acidic amino acids in the primary structure of nucleoplasmin (Kayne *et al.*, 1988). It is believed that nucleoplasmin plays a role in the process of storage, transport and deposition of histones onto DNA. Nucleoplasmin-like proteins were identified also in somatic tissues (Krohne & Franke, 1980; Cotten & Chalkley, 1987).

The karyophilic proteins N1 and N2, which are also abundant in nuclei of *Xenopus laevis* oocytes (Kleinschmidt & Franke, 1982), form complexes specifically with H3 and H4 (Kleinschmidt & Seiter, 1988). The amino acid sequence of N1 revealed two different acidic domains which exhibit distinct binding affinities to 'free' histones in solution on one hand, and to histones bound to nitrocellulose on the other. Interestingly, the histone binding does not seem to rely entirely on electrostatic interactions, as some residual histone binding was observed after deletion of both acidic domains (Kleinschmidt & Seiter, 1988). N1-like proteins also occur in nuclei of somatic tissues (Krohne, 1985).

The nonhistone high mobility group protein HMG-1 has, in addition to a high content of basic amino acids, an extended negatively charged region (Walker, 1982). HMG-1 favours the association of histones primarily into tetramers and acts as a nucleosome assembly factor *in vitro* (Bonne-Andrea *et al.*, 1984).

The SIR gene products (Nasmyth, 1982) and the *N*-terminal domain of histone H4 (Kayne *et al.*, 1988) are necessary for repression of the silent mating loci HMLα and HMRa in yeast. In the SIR3 protein a cluster of negative charges was identified which has some sequence similarities to an acidic domain of the karyophilic protein N1, and also to the acidic domain of nucleoplasmin (Kayne *et al.*, 1988).

A transcriptional activator of pseudorabies virus stimulates the binding of TFDII to a promoter sequence, when both factors compete with histones during nucleosome core particle formation (Workman *et al.*, 1988). It is conceivable that the stimulation is due to the 'negative blob' of the transcriptional activator which, by competing with DNA for histones, increases the probability of TFDII binding to the promoter site.

Some of the acidic nuclear proteins bind to histones in a highly specific manner and the function of the histone–nonhistone complexes seems to be related to the dynamics of histone–DNA interactions. In such processes the nonrandom use of acetylation positions could regulate the binding specificity at the *N*-terminal tail of the histone molecule, while the reversible nature of histone acetylation would provide the mechanism for the rapid dynamics intrinsic to the structural alterations of chromatin.

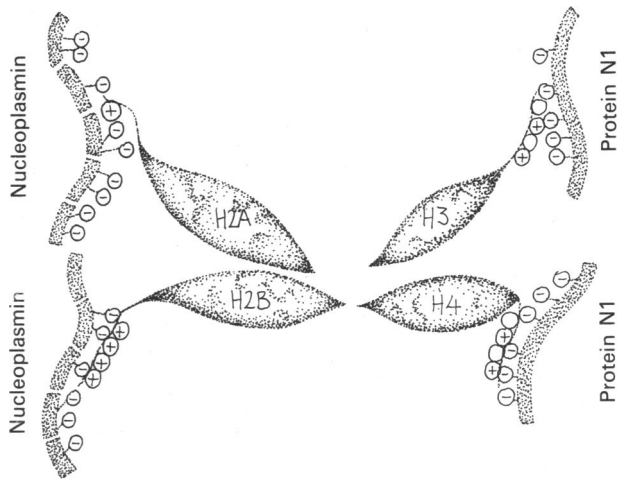

Fig. 3. Acidic nuclear proteins are required for histone organization and nucleosome formation

Core histones are bound to acidic nuclear proteins before their deposition on DNA. Nucleoplasmin specifically binds H2A and H2B, while the karyophilic protein N1 has a higher affinity for H3 and H4. Since newly synthesized core histones are reversibly acetylated before they bind to nucleoplasmin and protein N1, respectively, and become deacetylated after formation of nucleosomes, distinct patterns of histone acetylation appear to be involved in the interaction of histones with the acidic proteins of the nucleus. Histone acetylation can be expected to play a similar role in the case of transcriptionally displaced histones.

CONCLUDING REMARKS

The nonrandom use of acetylation positions correlated with different physiological situations strongly suggests that reversible histone acetylation has more than one function. The increased potential for specific interactions due to distinct patterns of acetylation disappears in the 'hyperacetylated' (fully acetylated) state of histones. Hyperacetylation of polynucleosomes leads to a net increase of the negative charge. However, because of the conserved character of the positions of acetylation and of the nucleosome core particle structure, acetylation of nucleosomal histones affects electrostatic interactions with a high degree of site specificity, and thus has to be related to a fundamental function of eukaryotic chromatin. In hyperacetylated nucleosomes a minor alteration of the nucleosome core particle configuration was detected, and possibly di- and tri-acetylated H4 is sufficient to cause such an alteration (Muller *et al.*, 1982). As hyperacetylation itself does not uncoil the higher order structure of chromatin, the *N*-terminal tails of core histones cannot play a major role in internucleosomal interactions of the condensed polynucleosomal configuration. However, some experiments suggest the possibility of an indirect control of the H1-binding site by acetylation of nucleosomal core histones (Stein, 1980;

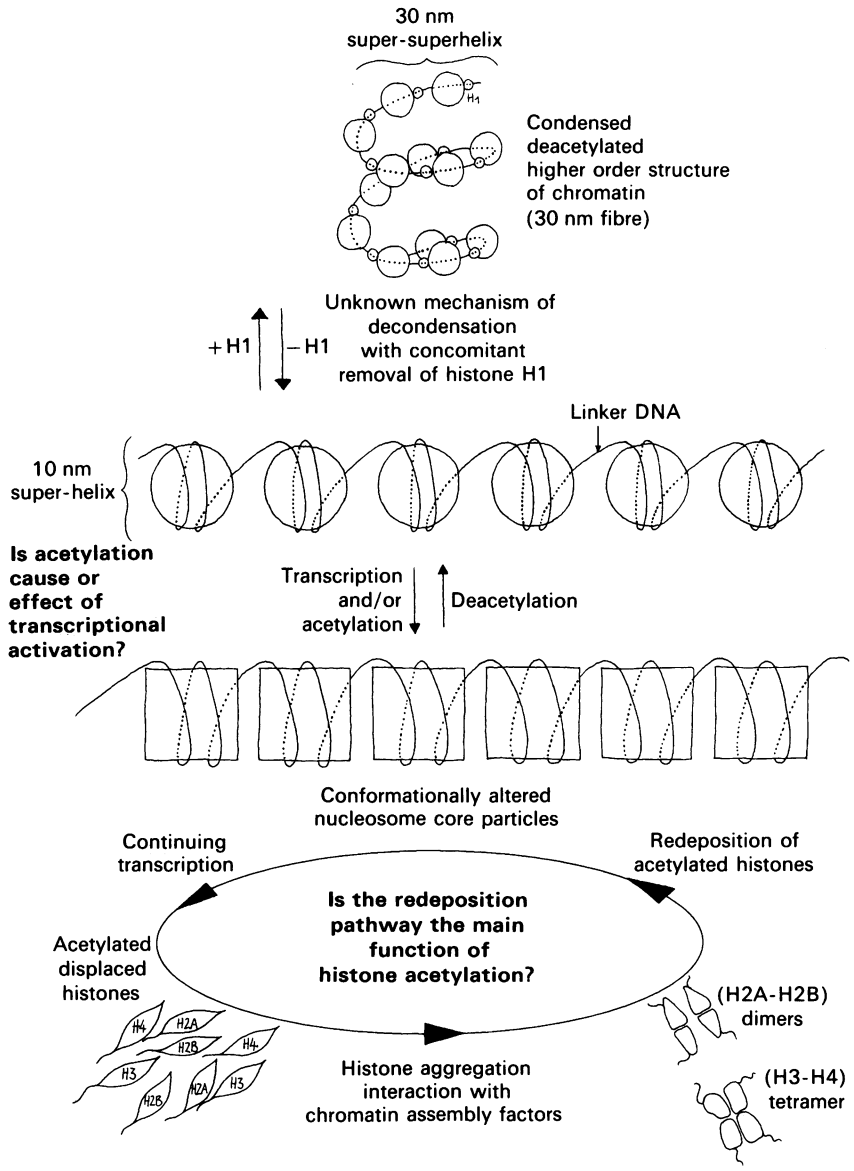

Fig. 4. Model illustrating a possible role of histone acetylation at the level of nucleosome assembly

Histone acetylation might be involved in the redeposition of transcriptionally displaced histones.

Allan *et al.*, 1982; Norton *et al.*, 1989). If so, then the assembly of the chromatosome might be the evolutionary constraint for the conserved character of acetylation positions, with especially high stringency in H3 and H4.

Paradoxically, there are two contrary effects of hyperacetylation. On the one hand, acetylation promotes histone aggregation (Prevelige & Fasman, 1987) and increases the efficiency of reconstitution of nucleosome core particles (Cotten & Chalkley, 1985; Norton *et al.*, 1989). On the other hand, hyperacetylated nucleosome core particles have a slightly relaxed conformation (Ausio & Van Holde, 1986), and at the late stage of spermatogenesis acetylation temporally precedes the displacement of histones (Christensen & Dixon, 1982; Christensen *et al.*, 1984). It is fair to conclude that the gradual increase in the degree of acetylation in the nucleosome core particle is concomitant with a gradual increase in the efficiency of nucleosome reconstitution of displaced histones (Cotton & Chalkley, 1985; Norton *et al.*, 1989), and possibly also concomitant with a gradual decrease in the binding affinity to histone H1. At the same time, acetylation might play a role in the control of transcription-driven supercoiling of DNA (Norton *et al.*, 1989; Giaever & Wang, 1988; Tsao *et al.*, 1989). These phenomena underline the multipurpose character of histone acetylation. Mechanisms involving topoisomerases and hypomethylation of DNA might be primarily responsible for the establishment of a relaxed chromatin structure which then leads as a secondary event to an altered pattern of acetylation with the potential for distinct functions. Topoisomerases are implicated in nucleosome removal from a promoter in yeast (Han *et al.*, 1988), and ubiquitous transcription factors bind to DNA apparently according to chromatin structure and/or methylation status of DNA (Becker *et al.*, 1987).

Histone acetylation appears to be involved in highly specific histone–protein interactions, as in yeast the *N*-

terminal domain of histone H4 is dispensable for growth but essential for repression of the silent mating loci; although in a mutant with deleted acetylation positions of H4 the silent mating loci are derepressed, other genes are repressible and inducible (Kayne *et al.*, 1988). Thus, the conformational change due to highly acetylated H4 detected in reconstituted nucleosome core particles (Muller *et al.*, 1982) cannot be in itself sufficient for transcriptional derepression.

Much attention has focused on the question whether and to what extent histone acetylation decreases the binding affinity of histones to DNA and thus favours displacement of histones. However, experiments with mononucleosomes *in vitro* have shown that there is need neither for histone displacement nor for histone acetylation to enable nucleosomal transcription *in vitro* (Lorch *et al.*, 1987, 1988; Losa & Brown, 1987; Workman & Roeder, 1987; Workman *et al.*, 1988), and transcriptional displacement of nucleosomal histones can occur without their preceding acetylation (Lorch *et al.*, 1987, 1988).

Newly synthesized histones H3 (Sealy & Chalkley, 1979) and H4 become reversibly acetylated before their deposition onto DNA (Louie & Dixon, 1972; Ruiz-Carrillo *et al.*, 1975; Jackson *et al.*, 1976). However, much is left to be clarified as to which enzyme, in which compartment and exactly when carries out the reversible acetylation of newly synthesized histones of the nucleosomes formed *de* novo. If acetylation is important for the deposition of newly synthesized histones (Louie & Dixon, 1972; Sealy & Chalkley, 1979), it could be just as important for the re-deposition of displaced 'old' histones. Thus, a main function of histone acetylation in eukaryotic transcription could be that of providing for nucleosomal histones to be displaced, the signal which later enables their 'soft landing' on DNA.

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